DOCTORAL THESIS

Development of Electrical Impedance Spectroscopy and Total Internal Reflection Microscopy Based Biosensing Assay Systems

Robin Ehrminger

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TAL TECH PRESS

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Declaration:
Hereby, I declare that this doctoral thesis, my original investigation, and achievement submitted for the doctoral degree at Tallinn University of Technology were not submitted for a doctoral or equivalent academic degree.

Robin Ehrminger

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signature

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Elektrilise impedantsi spektroskoopial ja täieliku sisepeegelduse fluorestsentsmikroskoopial põhinevad biotundlikud süsteemid

ROBIN EHRMINGER

TAL TECH
KIRJASTUS

Contents

List of publications

The list of author's publications, based on which the thesis has been prepared:

I Ojarand, J., Ehrminger, R., Min, M., Koel, A. "Compact Multichannel Device for Differential Impedance Spectroscopy of Microfluidic Sensors". Proc. 2018 16th Biennial Baltic Electronics Conference (BEC), October 8-10, 2018, Tallinn, Estonia: Piscataway, (NJ, USA) IEEE, 4 pp.
DOI: 10.1109/BEC.2018.8600955

II Ehrminger, R., Kopanchuk, S., Kivirand, K., Romann, T., Rinken, T., Min, M., Rinken, A. "Characterizing the bio-functionalization of gold surface with total internal reflection fluorescence (TIRF) microscopy." Proc. Est. Acad. Sci., vol. 69, pp. 27–34, 2020
DOI: 10.3176/proc.2020.1.02

III Laasfeld, T., Ehrminger, R., Thak, M. J., Kõlvart, K. R., Veisina, S., Min, M., Kopanchuk, S., Rinken A. "Budded baculoviruses as a receptor display system to quantify ligand binding with TIRF microscopy," Nanoscale, vol. 13, pp. 2436–2447, 2021
DOI: 10.1039/D0NR06737G

Abstract

Development of Electrical Impedance Spectroscopy and Total Internal Reflection Microscopy Based Biosensing Assay Systems

Biosensing systems detect biological analytes like pathogenic bacteria or viruses. These biosensing systems can be divided into two broad groups of instrumentations with various aspects and applications. The first one includes portable devices that are easy to use by non-specialists for mobile analysis. It focuses on biosensing systems that can be mass-produced at a low cost. The second one includes rather cost-intensive equipment that allows the accurate detection of biological interactions. The R&D efforts across the two aim to address critical aspects such as sensitivity, specificity, detection limits, linearity, environmental stability, scalability towards low-cost mass production, and high-throughput screening.

During this Ph.D. thesis, we have focused on implementing the first group of instrumentation in the form of validation of the mobile analyzer based on differential electrical impedance spectroscopy towards a mobile analysis of liquid samples. Our next aim was to develop a two-electrode biosensor and a flow chamber for low-cost biosensing applications with the aid of rapid prototyping. The conductive and semi-transparent thin-film electrodes were combined with a high-quality borosilicate coverslip as a base substrate. The surface of the gold film electrode was functionalized with different thiol-based self-assembled monolayers and characterized by total internal reflection fluorescence (TIRF) microscopy to display the specifically interacting antibodies with fluorescent labels. After the characterization of the functional surface, our next aim was the transfer and implementation to the 3D printed flow chamber for electrical impedance spectroscopy. However, the changes in the electric double-layer induced by the immobilization of *E. coli* bacteria and the baculovirus particles were found to require further research in several aspects. In particular, the measurement time and the differentiation between the analyte concentrations in the double-layer capacitance. The characterization of the electric double layer region was performed with TIRF microscopy. This method was significantly more sensitive, reliable, and environmentally friendly to display immobilized analytes, for instance, bacteria and viruses in the proximity of the surface.

Our further studies have focused on developing a new method to characterize ligand binding to membrane proteins using TIRF microscopy. Immobilizing budded baculovirus (insect virus) nanoparticles for microscopy studies is an innovative approach based on recombinant proteins in the natural membrane environment. The high sensitivity of the TIRF microscopy method enables a detailed display and analysis of ligand-binding processes, and the developed multiwell system permits medium content throughput for ligand screening. The method fills the gap between other averaging single-molecule techniques and live-cell microscopy assays. The baculovirus particles with the neuropeptide NPY_1 receptors were used as a model system to display the binding of tetramethylrhodamine (TAMRA) labeled high-affinity ligand UR-MC026 in TIRF microscopy. The binding affinities were found in good agreement with the validation assay in fluorescence anisotropy and previously published values. Furthermore, the system can detect kinetic events and quantify single receptor-ligand binding events combined with the single-molecule photobleaching technique.

Lühikokkuvõte

Elektrilise impedantsi spektroskoopial ja täieliku sisepeegelduse fluorestsentsmikroskoopial põhinevad biotundlikud süsteemid

Biotundlikud süsteemid on mõeldud selliste bioanalüütide tuvastamiseks nagu patogeensed bakterid ja viirused. Biotundlikke süsteeme saab vaadelda kahes laiemas kategoorias, millel on erinevad rakendused. Esimesse kategooriasse kuuluvad kehal kantavad seadmed mobiilsete analüüside tegemiseks, mida saab masstoodanguna valmistada väikeste kulutustega. Teise kategooria alla kuuluvad laboriseadmed, mis võimaldavad bioloogilisi kooslusi ja koos toimeid täpselt tuvastada. Mõlema kategooria uuringute ja arenduste eesmärk on parandada selliseid bioloogiliste osakeste identifitseerimise kriitilisi aspekte, nagu tundlikkus, spetsiifilisus, avastuspiirid, lineaarsus, stabiilsus keskkonna suhtes, mastaapsus masstootmise suunas ja võimelisus suure läbilaskevõimega sõeluuringuteks.

Doktoritöö keskendub alguses esimese kategooria seadmete täiustamisele, arendades nende analüüsivõimekust diferentsiaalsel elektrilisel impedants-spektroskoopial põhineva analüsaatori valideerimise näol vedelate proovide mobiilse analüüsi jaoks. Järgmine eesmärk oli kahe elektroodiga biosensori disainimise ja prototüüpimise põhimõtete väljatöötamine koos juurde kuuluvate kambrite tegemisega odavate biotundlike rakenduste jaoks.

Poolläbipaistvad õhukese juhtivkihiga elektroodid ühendati kvaliteetse boorsilikaadist katteklaasiga. Kullakile pind funktsionaliseeriti erinevate tiooli-põhiste isekujunevate monokihtidega, mida iseloomustati totaalse sisepeegeldusega fluorestsentsmikroskoopia (TIRF) abil tähistamaks spetsiifiliselt interakteeruvaid antikehi fluorestseeruvate märgistustega. Peale funktsionaalpinna pinna karakteriseerimist kujunes eesmärgiks elektrilise impedants-spektroskoopia ülekandmine 3D-trükitud voolukambrisse. Leiti, et *Escherichia coli* bakterite ja bakuloviiruse osakeste immobiliseerimisega põhjustatud elektrilise kaksikkihi omaduste ebastabiilsuse tõttu on vaja täiendavaid uuringuid. Eelkõige avaldus juhuslik ebastabiilsus mõõteajas ja seejärel ka analüüdi kontsentratsioonide eristamises kaksikkihi mahtuvuse kaudu. Elektrilise kaksikkihi iseloomustamine viidi läbi TIRF-mikroskoopia abil. Leiti, et TIRF-mikroskoopia meetod on oluliselt tundlikum ja usaldatavam, kui kuvada pinna lähedusse immobiliseeritud analüüte: baktereid või viiruseid.

Seetõttu keskendusid nimetatud teise kategooria seadmete uuringud uue meetodi väljatöötamisele selleks, et TIRF-mikroskoopiat kasutades iseloomustada ligandi seondumist membraanivalkudega. Bakuloviiruse (putukviiruse) nano-osakeste immobiliseerimine uuringuteks mikroskoopia kaudu on uus lähenemisviis, mis põhineb rekombinantsetel valkudel looduslikus membraanikeskkonnas. TIRF-mikroskoopia meetodi kõrge tundlikkus võimaldab ligandiga seondumise üksikasjalikku kuvamist ja analüüsi ning väljatöötatud mitmesüvendiline süsteem võimaldab keskmise kiirusega läbilaset sõelumise jaoks. See täidab tühimiku keskmiselt ühe molekuli kaupa toimivate meetodite ja elusrakkude mikroskoopiatestide vahel. Neuropeptiidi NPY1 retseptoritega bakuloviiruse osakesi kasutati mudelsüsteemina näitamaks tetrametüülrodamiiniga (TAMRA) märgistatud kõrge afiinsusega ligandi UR-MC026 seondumist TIRF-mikroskoopias. Seondafiinsused on heas kooskõlas fluorestsents-anisotroopia valideerimiskatsega ja varem avaldatud väärtustega. Süsteem tuvastab ka kineetilisi sündmusi ja kvantifitseerib ühe retseptor-ligand seondsündmusi koos ühe molekuli fotohelenduse tehnikaga.

Curriculum vitae

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